

Tissue oxygenation monitoring using resonance Raman spectroscopy during hemorrhage

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BACKGROUND: The ability to monitor the patient of hemorrhage noninvasively remains a challenge. We examined the ability of resonance Raman spectroscopy to monitor tissue hemoglobin oxygenation (RRS-StO₂) during hemorrhage and compared its performance with conventional invasive mixed venous (SmvO₂) and central venous (ScvO₂) hemoglobin oxygen saturation as well as with near-infrared spectroscopy tissue hemoglobin oxygenation (NIRS-StO₂).

METHODS: Five male swine were anesthetized and instrumented followed by hemorrhage at a rate of 30 mL/min for 60 minutes. RRS-StO₂ was continuously measured from the buccal mucosa, and NIRS-StO₂ was continuously measured from the forelimb. Paired interval measures of SmvO₂, ScvO₂, and lactate were made. Pearson correlation was used to quantify the degree to which any two variables are related. Receiver operating characteristic (ROC) area under the curve values were used for pooled data for RRS-StO₂, NIRS-StO₂, SmvO₂, and ScvO₂ to compare performance in the ability of tissue oxygenation methods to predict the presence of an elevated arterial blood lactate level.

RESULTS: Sequential RRS-StO₂ changes tracked changes in SmvO₂ ($r = 0.917$; 95% confidence interval [CI], 0.867–0.949) and ScvO₂ ($r = 0.901$; 95% CI, 0.828–0.944) during hemorrhage, while NIRS-StO₂ failed to do so for SmvO₂ ($r = 0.283$; 95% CI, 0.04919–0.4984) and ScvO₂ ($r = 0.142$; 95% CI, 0.151 to 0.412). ROC curve performance of oxygenation measured to indicate lactate less than or greater than 3 mM yielded the following ROC area under the curve values: SmvO₂ (1.0), ScvO₂ (0.994), RRS-StO₂ (0.972), and NIRS-StO₂ (0.611).

CONCLUSION: RRS-StO₂ seems to have significantly better ability to track central oxygenation measures during hemorrhage as well as to predict shock based on elevated lactate levels when compared with NIRS-StO₂. (*J Trauma Acute Care Surg.* 2014;76: 402–408. Copyright © 2014 by Lippincott Williams & Wilkins)

KEY WORDS: Hemorrhagic shock; tissue hemoglobin oxygen saturation; resonance Raman spectroscopy; near-infrared spectroscopy; noninvasive monitoring; swine.

Detecting the presence of shock, its severity, and the adequacy of resuscitations continue to be high priorities in the development of technologies for the care of trauma patients. Because of the rapid echelons of care that a trauma patient passes through including the prehospital setting, noninvasive monitoring technologies are appealing. It has been argued that technologies designed to monitor for evidence of hypoxia at the tissue level would provide the most value to help prevent underresuscitation and overresuscitation.¹ To this end, technologies such as gastric tonometry, sublingual tonometry, transcutaneous gas measurement, near-infrared spectroscopy, and others have been studied.^{2–7} Each of these relies on principles of microcirculatory oxygen transport and the fact that the postextraction compartment of tissue is dominated by venous

blood. While each has advantages, none has been uniformly accepted, and each has the potential to be confounded.

We are exploring the use of resonance Raman spectroscopy (RRS) as a method to monitor tissue oxygenation. RRS has been valuable in the structural and ligand binding assessment of heme proteins.⁸ The vibrational bands of heme are well-known but have only recently been explored to provide medically relevant information.^{9,10} The spectroscopic basis for the application of RRS of hemoglobin lies in the resonance vibrational enhancement of hemoglobin in the deep violet region, allowing simultaneous identification and monitoring of the proportion of oxy and deoxy species of hemoglobin in a concentration-dependent manner with a single wavelength of light.¹¹ This produces a chemical fingerprint of the species with little or no interference from other compounds in the tissue being interrogated (Fig. 1).^{11–13}

In this pilot study, we hypothesized that RRS would detect changes in buccal mucosal hemoglobin oxygen saturation (RRS-StO₂) in response to hemorrhage and that it would track changes in mixed and central venous hemoglobin oxygen saturation. Furthermore, we hypothesized that it would perform as well or better in this regard as compared with the use of near-infrared spectroscopy–derived tissue hemoglobin oxygen saturation (NIRS-StO₂).

MATERIALS AND METHODS

The Virginia Commonwealth University Institutional Animal Care and Use Committee approved this study, which

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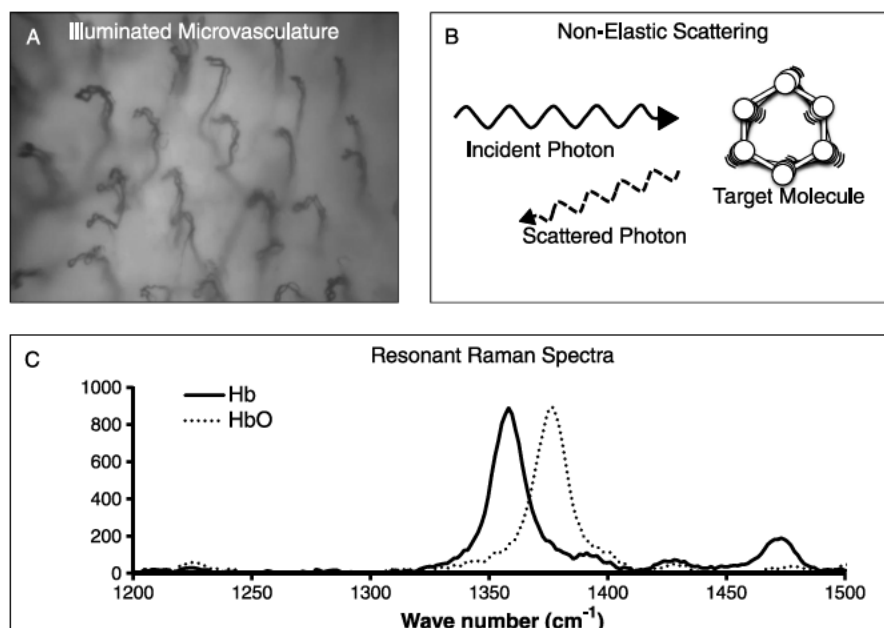


Figure 1. A, Example of oral microvasculature being interrogated by RRS (see text for details). B, Depiction of Raman vibrational spectroscopy phenomena. C, Example of resonant Raman spectra of oxyhemoglobin and deoxyhemoglobin using excitation at 405 nm.

adhered to the Guide for the Care and Use of Laboratory Animals (National Research Council, revised 2011).

Five male Yorkshire swine with a mean (SD) weight of 38 (1.5) kg were used in the study. Animals were fed a standard swine diet and were given one half of their morning feed ration on the morning of the experiment. Animals were sedated with intramuscular mixture of ketamine (20 mg/kg) and xylazine (2 mg/kg) before anesthesia induction. Anesthesia was induced with 4-mg/kg to 8-mg/kg intravenously administered propofol and maintained with intravenous α -chloralose (40–50-mg/kg intravenous bolus followed by 10-mg/kg/h infusion). Animals were intubated and ventilated (Fabius GS, Draeger Medical Inc., Telford, PA) with a tidal volume of 8 mL/kg to 10 mL/kg and 0.3 FIO₂. Ventilation was adjusted to produce an end-tidal CO₂ value of 38 mmHg to 44 mmHg (Biopac Systems Inc., Goleta, CA). Ventilation was unchanged after baseline was established and for the duration of the experiment. Core temperature of 38°C to 38.5°C was maintained through feedback via a rectal temperature probe and heating blanket (Blanketrol II, Cincinnati Sub-Zero Products, Cincinnati, OH).

Continuous electrocardiogram monitoring was performed using a standard 3-lead configuration (Biopac Systems Inc.). Mean arterial pressure (MAP) and arterial blood sampling was performed via an arterial catheter surgically placed in the carotid artery. A pulmonary artery catheter (Swan-Ganz Catheter, 746F8, Edwards Lifesciences, Irvine, CA) was inserted into the pulmonary artery via the right external jugular vein for monitoring of mixed venous hemoglobin oxygen saturation (SmvO₂) and central venous hemoglobin oxygen saturation (ScvO₂). The left external jugular vein was cannulated for fluid administration. The left femoral artery was exposed and

cannulated for subsequent controlled arterial hemorrhage using a 9 Fr catheter (Smith Medical, Dublin, OH).

Tissue oxygen saturation measurements (StO₂) were performed using two technologies. (1) The microvascular oximeter (Pendar Medical, Cambridge, MA) uses RRS to determine relative concentrations of oxyhemoglobin and deoxyhemoglobin. A 405-nm, 4-mW laser coupled to a complex plastic fiber-optic cable illuminates an area of tissue approximately 3 mm in diameter and less than 1 mm deep. An autofluorescence imaging system using the same wavelength was used to take images of the oral mucosa from one of the authors (D.V.) to demonstrate an example of the type of oral microvasculature being interrogated with RRS (Fig. 1). The laser light excites oxyhemoglobin and deoxyhemoglobin molecules into distinct vibrational states resulting in a differential wavelength shift of the scattered light (Fig. 1). The spectrum of the scattered light is captured in the device's charged-coupled device-based spectrometer each second, with distinct sharp peaks linearly proportional to the concentration of oxyhemoglobin and deoxyhemoglobin. The spectral peaks are used to calculate the percentage of tissue oxygen saturation (StO₂) as the relative ratio of concentration of oxygenated hemoglobin to that of total hemoglobin.^{11–13} To increase precision of the result, 20 to 80 spectra are averaged. The hemoglobin oxygen saturation (%) value is updated on the integrated monitor each second. The RRS-StO₂ sensor was placed on the buccal mucosa.

(2) NIRS (INVOS, Covidien, Mansfield, MA). The device uses a range of light frequencies between 650 nm and 1,100 nm. At these wavelengths, tissue is translucent. In addition, at these wavelengths oxyhemoglobin and deoxyhemoglobin differentially absorb light.¹⁴ The technique is used in a reflectance mode in which the light source and detectors are spaced

TABLE 1. Average Baseline Characteristics of Animals

Baseline	Weight, kg	Blood Volume mL	Heart Rate, beats/min	MAP, mmHg	Hemoglobin, g/dL	Lactate, mmol/L	SmvO ₂ , %	ScvO ₂ , %	RRS-StO ₂ , %	NIRS-StO ₂ , %
Mean	38.4	2,496	109	98	8.8	0.7	60.8	64.4	62.0	53.4
SD	1.5	98.6	6.6	20.7	1.2	0.2	4.7	4.0	4.7	7.0

to allow the differential absorption spectra of oxyhemoglobin and deoxyhemoglobin to be obtained 2 cm below the surface while excluding signal from more shallow tissue. The device continuously samples spectra and updates values every 5 seconds. The NIRS sensor was placed over the left upper forelimb of the animal.

Once instrumentation was completed, animals were monitored for a 20-minute baseline period. At the end of baseline, hemodynamics, SmvO₂, ScvO₂, RRS-StO₂, and NIRS-StO₂ were collected as were arterial lactate and hemoglobin oxygen saturation (SaO₂) (ABL800, Radiometer America, Westlake, OH).

Five minutes after baseline measurement, controlled hemorrhage was started through the femoral artery at a rate of 30 mL/min (Masterflex Pump; Cole Parmer Instruments, Court Vernon Hills, IL). Baseline parameters described previously were recorded every 5 minutes for 60 minutes. At 60 minutes, animals were euthanized with potassium chloride (2 mEq/kg).

Statistical Analysis

Descriptive statistics are expressed as means and SDs. Pearson correlation was used to quantify the degree to which any two variables are related. We used unpaired *t* test to quantify differences between the variables. Where necessary, 95% confidence intervals (CIs) are included. Summary statistics using receiver operating characteristic (ROC) area under the curve (AUC) values were used for pooled data for RRS-StO₂, NIRS-StO₂, SmvO₂, and ScvO₂ to compare performance in the ability of tissue oxygenation methods to predict the presence of elevated lactate levels. Linear regression was used to quantify the relationships between variables to account for the repeated-measures structure of the data. Data analysis was performed on GraphPad Prism6 (GraphPad Software, Inc., La Jolla, CA).

RESULTS

Five animals were studied. Tables 1 and 2 list the mean and SD of baseline and end-of-hemorrhage values for each oxygenation indicator as well as major hemodynamic variables. Sequential regional RRS-StO₂ changes tracked changes in SmvO₂ ($r = 0.917$; 95% CI, 0.867–0.949) and ScvO₂ ($r = 0.901$; 95% CI, 0.828–0.944) during hemorrhage, while NIRS-StO₂ failed to do so for SmvO₂ ($r = 0.283$; 95% CI, 0.04919–0.4984)

and ScvO₂ ($r = 0.142$; 95% CI, –0.151 to 0.412). Scatter plots (Fig. 2) demonstrate the relationship between the variables. There were significant differences between the correlation coefficients when comparing RRS-StO₂ and NIRS-StO₂ with SmvO₂ (0.917 and 0.283, $p < 0.0001$) and when comparing with ScvO₂ (0.901 and 0.142, $p < 0.0001$). Unpaired *t* tests revealed no significant difference between RRS-StO₂ and both SmvO₂ and ScvO₂ ($p > 0.05$), whereas it indicated a significant difference between NIRS-StO₂ and both SmvO₂ and ScvO₂ ($p < 0.0001$).

ROC AUC (Fig. 3) was used to compare the ability of the various oxygenation monitoring methods to detect shock based on lactate levels. Lactate levels equal to or greater than 3 mM and equal to or greater than 4 mM were used to distinguish between shocked and nonshocked states. ROC yielded the following AUC values for lactates equal to or greater than 3 mM; SmvO₂ (1), ScvO₂ (0.994), RRS-StO₂ (0.972), and NIRS-StO₂ (0.611). When used to distinguish between lactate levels equal to or greater than 4 mM, AUC values were as follows: SmvO₂ (0.994), ScvO₂ (0.998), RRS-StO₂ (0.952), and NIRS-StO₂ (0.589) (Fig. 4). Nearly identical performance was noted when lactate thresholds of 5 mM and 6 mM were used.

Linear regression comparing RRS-StO₂ and SmvO₂ yielded a slope of 1.01 (95% CI, 0.892–1.11) with a Y intercept at 6.99 (95% CI, 3.366–10.62), meaning that when SmvO₂ equals zero, RRS-StO₂ will equal 6.99. A linear regression comparing NIRS-StO₂ and SmvO₂ yielded a slope of 0.126 (95% CI, 0.021–0.229) with a Y intercept at 44.96 (95% CI, 41.49–48.44), meaning that when SmvO₂ equals zero, NIRS-StO₂ will equal 44.96. There was a significant difference between the slopes ($F = 134.48$, $p < 0.0001$). Nearly identical results were obtained when the same analysis was performed comparing RRS-StO₂ and NIRS-StO₂ with ScvO₂.

DISCUSSION

Rapidly detecting the presence of impending shock, its degree when present, and its resolution during treatment continue to be high priority goals in the field of critical care. While invasive approaches using oxygen transport parameters such as SmvO₂ and ScvO₂ are valuable, their invasive nature makes their use in earlier echelons of care problematic.^{15–18} Noninvasive technologies, which can be easily applied and

TABLE 2. Average End-of-Hemorrhage Characteristics of Animals and Average Total Blood Loss

End of Hemorrhage	Hemorrhage Volume, %	Heart Rate, beats/min	MAP mmHg	Hemoglobin, g/dL	Lactate, mmol/L	SmvO ₂ , %	ScvO ₂ , %	RRS-StO ₂ , %	NIRS-StO ₂ , %
Mean	66.3	172	22	7.5	11.2	5.6	7.6	6	48
SD	7.9	58	9.97	2.4	0.98	1.64	2	1.7	12.28

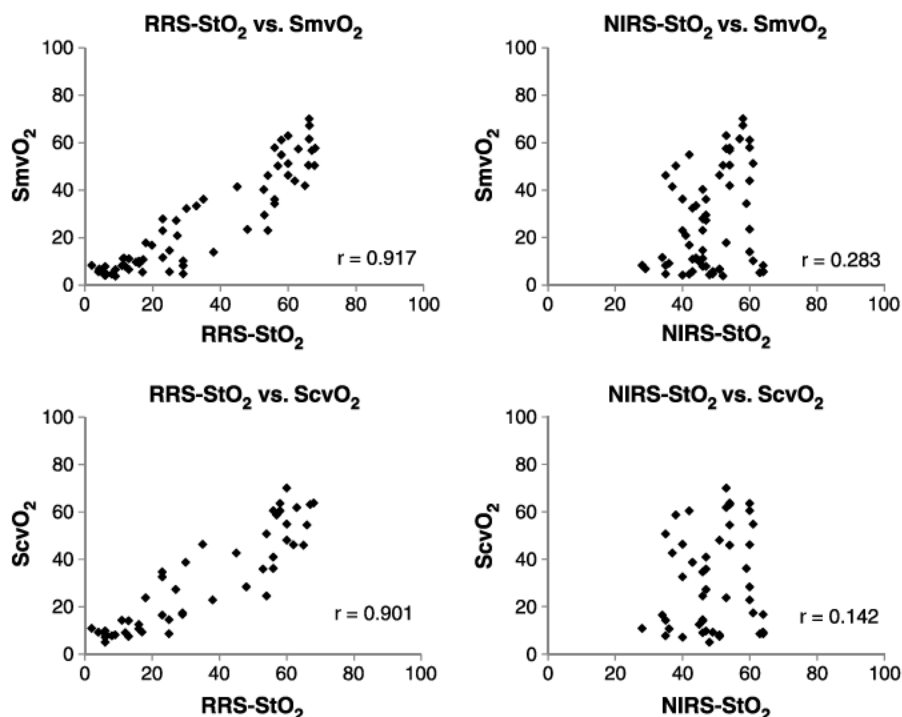


Figure 2. Scatter plots and Pearson correlations comparing RRS-StO₂ and NIRS-StO₂ with SmvO₂ and ScvO₂ (see text for details).

allow for continuous monitoring, would potentially offer significant value.

In this swine model of hemorrhagic shock, RRS-StO₂ of the oral mucosa tracked simultaneous changes in ScvO₂ and SmvO₂ statistically and clinically significantly better compared with NIRS. In addition, when compared with an elevated lactate as an independent marker of shock, the ability of RRS-StO₂ to detect a change was superior to NIRS-StO₂. Because RRS-StO₂ and NIRS-StO₂ are both local measures of tissue oxygenation versus global tissue aggregate measures (SmvO₂ and ScvO₂), we felt that comparing these values to a “shared” metabolic link would assist in evaluating their respective performance. Furthermore, because of the biphasic relationship of oxygen delivery and oxygen consumption, lactate levels are

not elevated until a critical level of oxygen delivery and tissue oxygen extraction is reached.¹⁹ Because animals have greater physiologic reserve than humans do, lactate levels are not elevated until SvO₂ levels drop to less than 40%. This study confirms our previous work in small animals and in vitro settings demonstrating the accuracy of RRS to measure hemoglobin oxygen saturation over the entire range of clinically relevant hematocrit and hemoglobin oxygen saturation levels and similar performance of RRS-StO₂ to ScvO₂ in signaling elevated lactate levels.^{12,20}

The Raman effect is a measurable loss or gain of energy of scattered photons that correspond to the vibrational energies of a molecule in response to excitation from a light source at a precise wavelength (Fig. 1).⁸ This type of vibrational spectroscopy

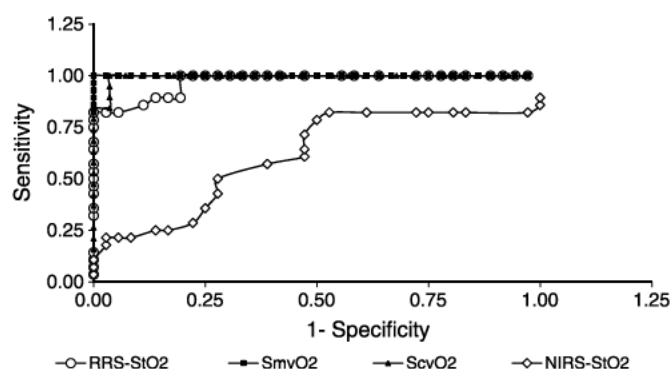


Figure 3. ROC AUC comparing the performance of SmvO₂, ScvO₂, RRS-StO₂, and NIRS-StO₂ to discriminate between shocked and nonshocked animals based on lactate levels equal to or greater than 3 mM.

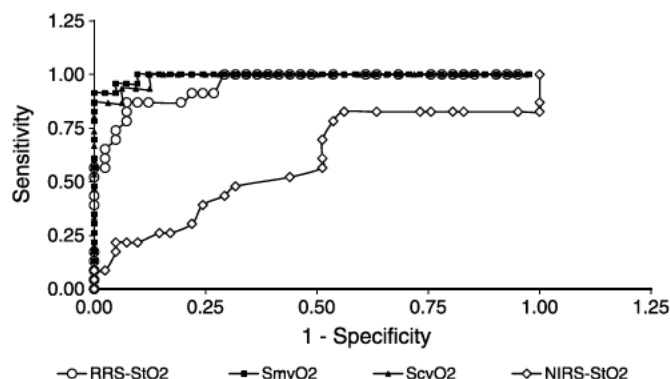


Figure 4. ROC AUC comparing the performance of SmvO₂, ScvO₂, RRS-StO₂, and NIRS-StO₂ to discriminate between shocked and nonshocked animals based on lactate levels equal to or greater than 4 mM.

is fundamentally different from the differential absorption spectroscopy (the basis for NIRS).¹⁴ While Raman scattering is a low-intensity phenomenon requiring sensitive detectors, the signal is greatly enhanced when the excitation energy is in resonance with the electronic transition of the species being interrogated. This allows for the selective detection of very low concentrations of a molecular species in a complex mixture. In this study, the 405-nm wavelength used is near the peak for the heme Soret band, thus allowing for the production of strong resonance spectra of oxyhemoglobin and deoxyhemoglobin.¹¹ This resonance results in an enhanced signal-to-noise ratio.

Reasons for the improved performance of RRS-StO₂ over NIRS-StO₂ may in part be based on the contamination of the NIRS-StO₂ signal with myoglobin.¹⁴ The NIRS spectra of oxymyoglobin and deoxymyoglobin are indistinguishable from that of oxyhemoglobin and deoxyhemoglobin. Studies have dealt with this issue by adding the species together (HbO₂ + MbO₂) and (Hb + Mb). Thus StO₂ as determined by NIRS (which include signal from muscle) would be determined as (HbO₂ + MbO₂) / (HbO₂ + MbO₂ + Hb + Mb). Awareness of this is important for several reasons including the fact that the P50 of myoglobin is 5 mmHg as opposed to 27 mmHg for hemoglobin and is thus fully saturated under most conditions.^{21,22} In addition, in human skeletal muscle, myoglobin and hemoglobin will exist in approximately equal concentrations.^{14,23,24} In the setting of hemorrhage, the fractional signal from myoglobin may even be increased during resuscitation, as hematocrit decreases during crystalloid resuscitation. Evidence using proton magnetic resonance (which is able to distinguish deoxy Mb from deoxy Hb) indicates that myoglobin might account for the majority of the NIRS signal.^{25,26} Other confounders such as pigment and fat may explain why baseline values in volunteers can widely vary.²⁷ It may also explain why NIRS has not shown more sensitivity and specificity over blood pressure and lactate as an earlier warning system in either the setting of trauma or sepsis and why some are attempting to use dynamic changes in NIRS produced in response to vaso-occlusion to gain additional information.^{6,27-29} Recent studies have examined the performance of commercially available NIRS devices on humans at baseline and in response to ischemic occlusion including the device used in this study. These studies demonstrate significant differences in performance between devices in baseline readings and in response to ischemia as well as significant differences in values at different sites including differences in repeatability at the same site with the same device.³⁰⁻³²

At the wavelength used for RRS-StO₂ measurement, the expected depth of penetration is less than 1 mm and is not contaminated by muscle, pigment, or fat in the buccal tissues. Previous studies using RRS on the sublingual surface of the tongue noted no contribution of tongue myoglobin from the tongue mucosa site after performing saline perfusion of the tongue.²⁰ The oral mucosa as shown in this study and others seems to be very sensitive to changes in perfusion.³³⁻³⁵ However, because the buccal mucosa receives its blood supply from branches of the internal carotid artery and its microvascular orientation is more looped, it could be argued that it would not be an appropriate site of monitoring.³⁶ Furthermore, since RRS-StO₂ is only a reflection of the balance between

mucosal blood flow and metabolism, it could be argued that the oral mucosa would not be metabolically active enough to reflect the global aggregates of tissue oxygenation such as ScvO₂ and SmvO₂. We have previously demonstrated that the StO₂ in the sublingual mucosa of rats completely desaturates in less than 60 seconds after cardiac arrest.²⁰ It was thus essential in this study to follow ScvO₂ and SmvO₂ to their lowest obtainable values and compare buccal mucosa RRS-StO₂ values with them. The very low values of RRS-StO₂ and their correlation to low ScvO₂ and SmvO₂ values supports this as an attractive tissue site for interrogation.

While it could be argued that the values of ScvO₂, SmvO₂, and RRS-StO₂ less than 40% are not relevant, clinical studies in heart failure and sepsis have demonstrated humans with values well less than 40% accompanied by elevated lactate levels.^{15,16,37} Although values this low have not been reported in human traumatic hemorrhage, this may be caused by the fact that patients are rarely instrumented and monitored quickly before resuscitation. The ability to track such low values might also provide value in monitoring cardiac arrest resuscitation where values would be expected to be lower.³⁸

In addition to the small number of animals, this study has several limitations. We tested only one manufacturer's brand of NIRS monitoring. While there are several other manufacturers of this technology for monitoring peripheral tissue perfusion of the skeletal muscle, all are based on similar principals, with their differences being mainly where the suggested site of monitoring is and their algorithmic approach to signal analysis.¹⁴ Therefore, the use of other devices may have yielded different NIRS performance. However, as indicated earlier, performance between devices have been noted to be significantly different and potentially not comparable with each other even in the same individual.³⁰⁻³² We monitored only at one site (proximal forelimb). This area in our animals lacked redundancy of skin and fat and was believed to be suitable for reproducibility. While others have used the hind limb, there should not be a significant difference from an anatomic standpoint since the animals are quadrupeds. However, we did desire not to confound NIRS-StO₂ with potential changes in blood flow to the hind limb caused by the placement of the 9 Fr (61 cm) femoral artery catheter used to hemorrhage since the tip of this catheter lies at the bifurcation of the abdominal aorta. Still, others have used the hind limb (with different NIRS technology), so we cannot rule out a difference in performance had we chosen another site or NIRS device.³⁹ We did not resuscitate animals, so we cannot report on the performance of either RRS-StO₂ or NIRS-StO₂ to track changes during resuscitation. We felt, however, that it was essential to demonstrate the ability of RRS to track StO₂ to its lowest levels during exsanguinating levels of hemorrhage as microvascular levels of hemoglobin drop and to prove that the oral mucosa would reflect central oxygenation. We note that there is a lack of such studies using NIRS.

Lastly, our baseline ScvO₂ and SmvO₂ were lower than might be expected. This may be caused by the fact that young swine are highly susceptible to iron-deficient anemia secondary to low tissue stores at birth and extremely rapid growth.⁴⁰ The swine we used did not receive iron supplementation in their diet.

CONCLUSION

In this model of hemorrhagic shock, RRS-StO₂ tracked changes in SmvO₂ and ScvO₂ well and seemed to have similar discriminatory power of SmvO₂ and ScvO₂ to detect shock based on lactate levels. Use of NIRS-StO₂, however, did not track SmvO₂ and ScvO₂ during hemorrhage and demonstrated less ability to discriminate shock states as compared with RRS-StO₂. While RRS-StO₂ may be a promising technique for the noninvasive evaluation of hemorrhage, additional testing will be required including its ability to track changes in tissue oxygenation during resuscitation.

AUTHORSHIP

M.H.T. and K.R.W. designed this study. M.H.T., G.T.D., and K.R.W. collected and analyzed the data. All authors contributed to data interpretation. M.H.T. and K.R.W. wrote the manuscript. M.H.T., R.W.B., J.T., I.T.F., P.R., D.V., and K.R.W. contributed to critical revision.

DISCLOSURE

K.R.W., R.W.B., J.T., and I.T.F. have intellectual property on the use of RRS for tissue oxygenation monitoring assigned to Virginia Commonwealth University. P.R. and D.V. are officers in Pendar Medical, which have licensed the Raman spectroscopy technology from Virginia Commonwealth University.

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